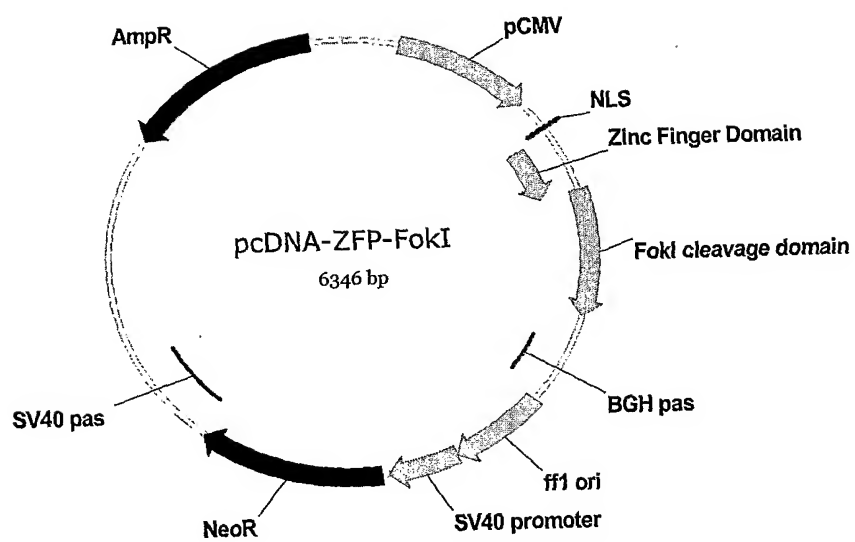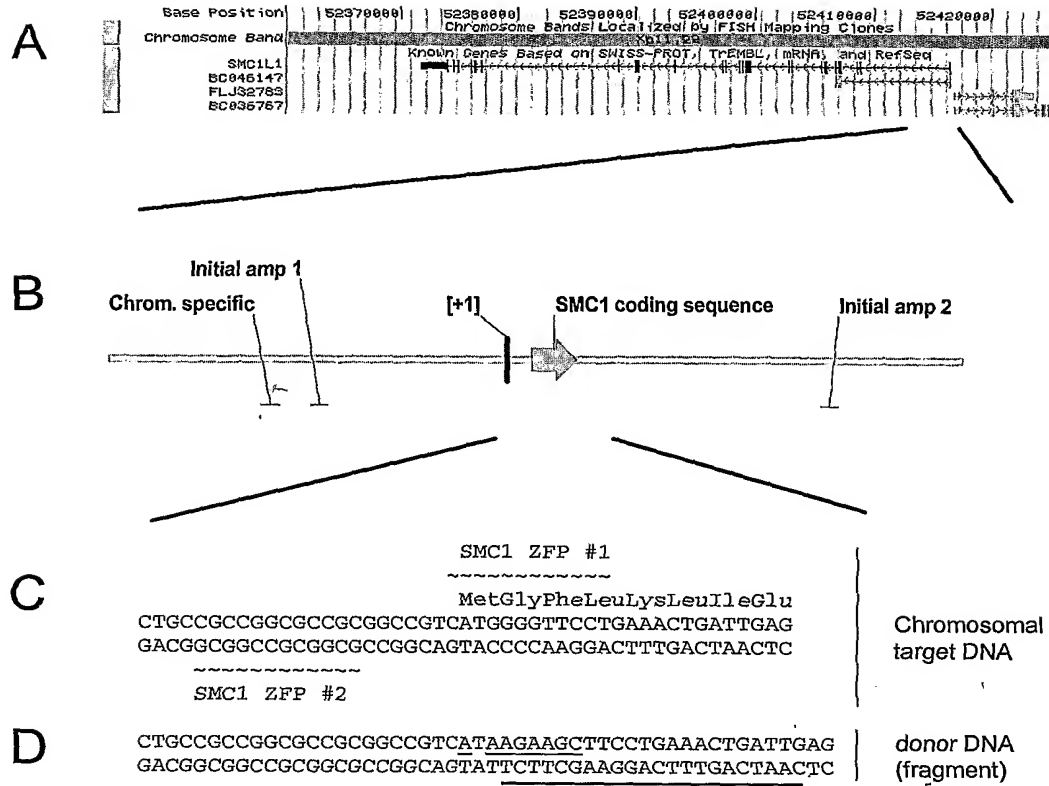# FIGURE 1

```
                        MetGlyPheLeuLysLeuIle    (SEQ ID NO:2)
CTGCCGCCGGCGCCGCGGCCGTCATGGGGTTCCTGAAACTGATT
GACGGCGGCCGCGGCGCCGGCAGTACCCCAAGGACTTTGACTAA    (SEQ ID NO:1)
     |      |      |      |      |    . |      |      |
     5     10     15     20     25     30     35     40
```
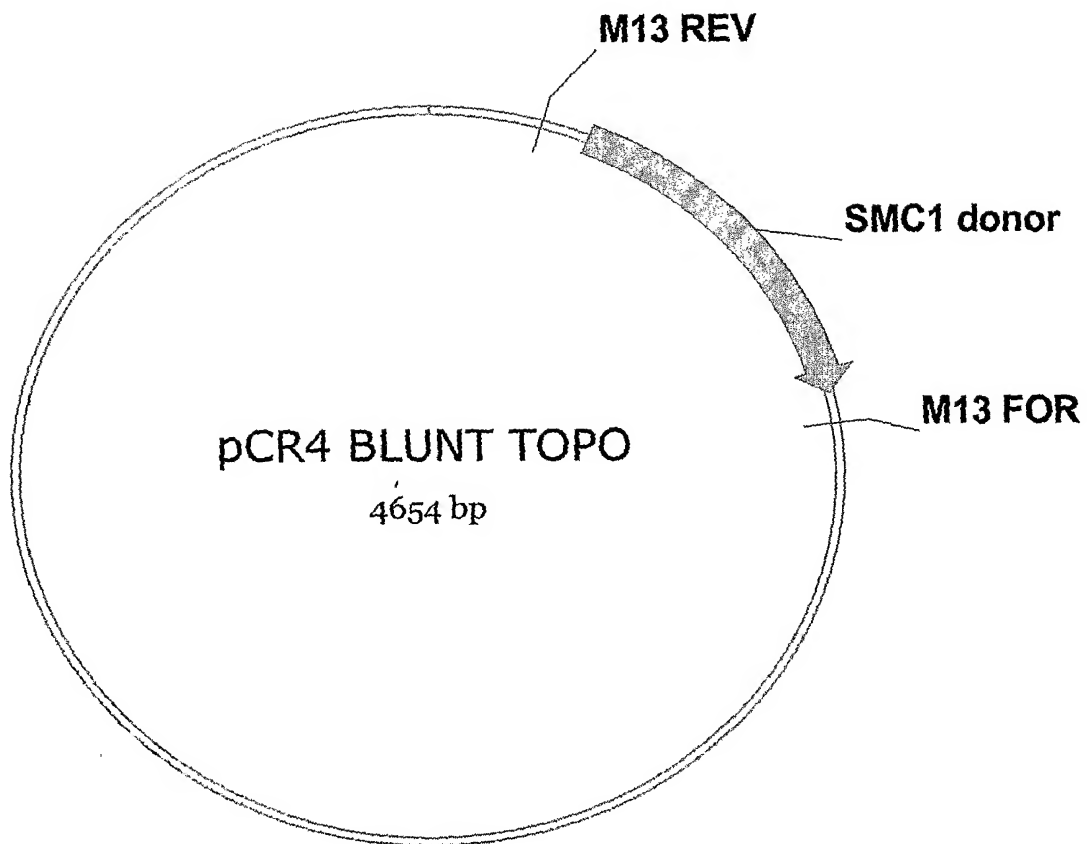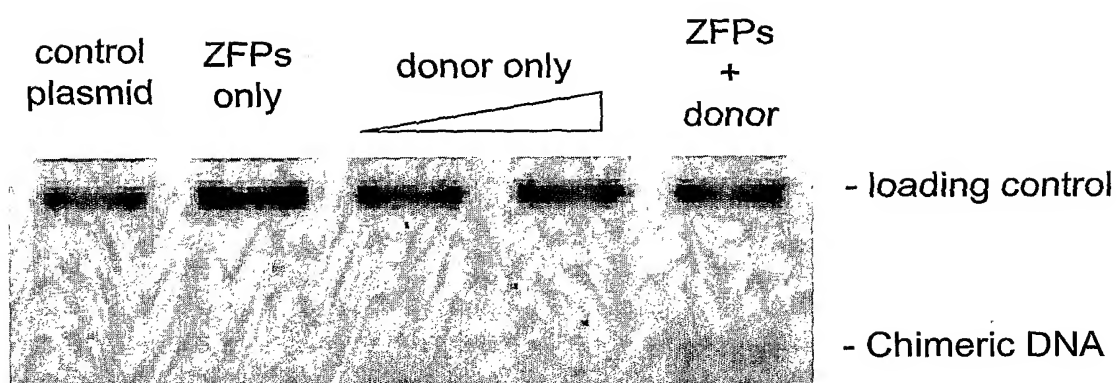
FIGURE 2

FIGURE 3

A

Base Position | 52370000 | 52380000 | 52390000 | 52400000 | 52410000 | 52420000

Chromosome Band | Chromosome Bands Localized by FISH Mapping Clones

Known Genes Based on SWISS-PROT, TrEMBL, mRNA and RefSeq

SMC1L1
BC046147
FLJ32783
BC036757

B

Initial amp 1

Chrom. specific   [+1]   SMC1 coding sequence   Initial amp 2

C

```
                        SMC1 ZFP #1
                        ~~~~~~~~~~~~
                        MetGlyPheLeuLysLeuIleGlu
CTGCCGCCGGCGCCGCGGCCGTCATGGGGTTCCTGAAACTGATTGAG
GACGGCGGCCGCGGCGCCGGCAGTACCCCAAGGACTTTGACTAACTC
                    ~~~~~~~~~~~~
                    SMC1 ZFP #2
```

Chromosomal
target DNA

D

```
CTGCCGCCGGCGCCGCGGCCGTCATAAGAAGCTTCCTGAAACTGATTGAG
GACGGCGGCCGCGGCGCCGGCAGTATTCTTCGAAGGACTTTGACTAACTC
```

donor DNA
(fragment)

FIGURE 4
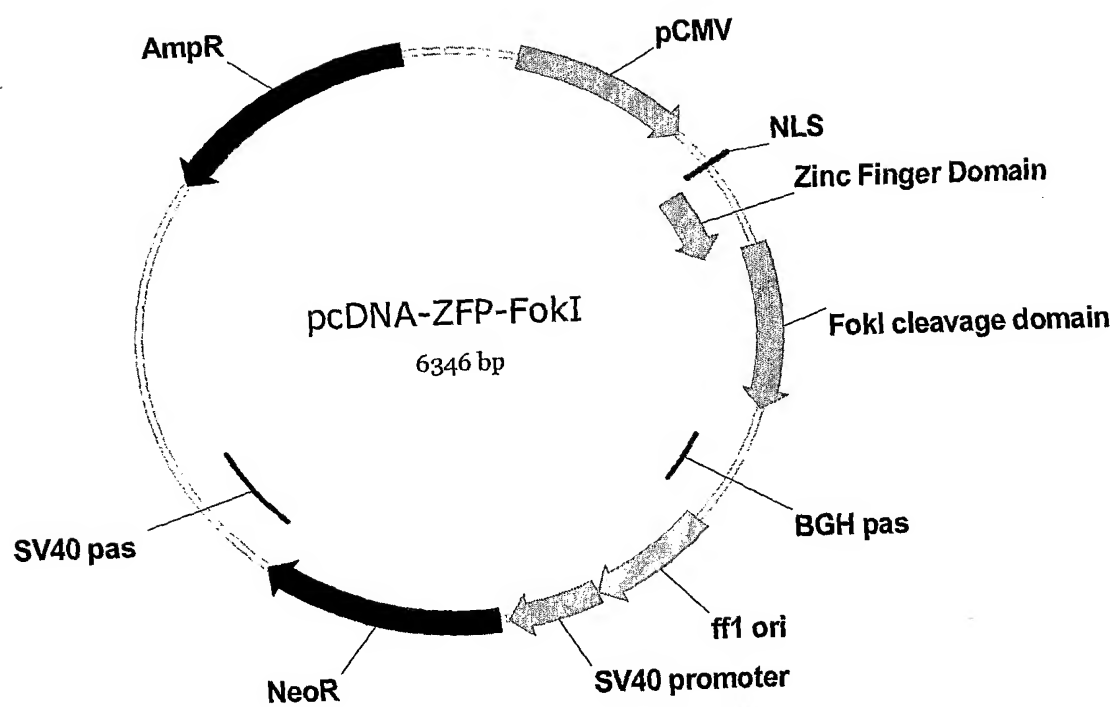
FIGURE 5

FIGURE 6

```
TAGTCCTGCAGGTTTAAACGAATTCGCCCTTCTCAGCAAGCGTGAGCTCA    50
GGTCTCCCCCGCCTCCTTGAACCTCAAGAACTGCTCTGACTCCGCCCAGC    100
AACAACTCCTCCGGGGATCTGGTCCGCAGGAGCAAGTGTTTGTTGTTGCC    150
ATGCAACAAGAAAAGGGGGCGGAGGCACCACGCCAGTCGTCAGCTCGCTC    200
CTCGTATACGCAACATCAGTCCCCGCCCCTGGTCCCACTCCTGCCGGAAG    250
GCGAAGATCCCGTTAGGCCTGGACGTATTCTCGCGACATTTGCCGGTCGC    300
CCGGCTTGCACTGCGGCGTTTCCCGCGCGGGCTACCTCAGTTCTCGGGCG    350
TACGGCGCGGCCTGTCCTACTGCTGCCGGCGCCGCGGCCGTCATaagaag    400
cTTCCTGAAACTGATTGAAGGGCGAATTCGCGGCCGCTAAATTCAATTCG    450
CCCTATAGTGAGT
```

(SEQ ID NO: 6)

FIGURE 7

```
                              TyrLysAsnSerAspAsnAspLysVal      (SEQ ID
NO:8)
CTTCCAACCTTTCTCCTCTAGGTACAAGAACTCGGATAATGATAAAGTCC
GAAGGTTGGAAAGAGGAGATCCATGTTCTTGAGCCTATTACTATTTCAGG      (SEQ ID
NO:7)
      |    |    |    |    |    |    |    |    |    |
      5   10   15   20   25   30   35   40   45   50
```
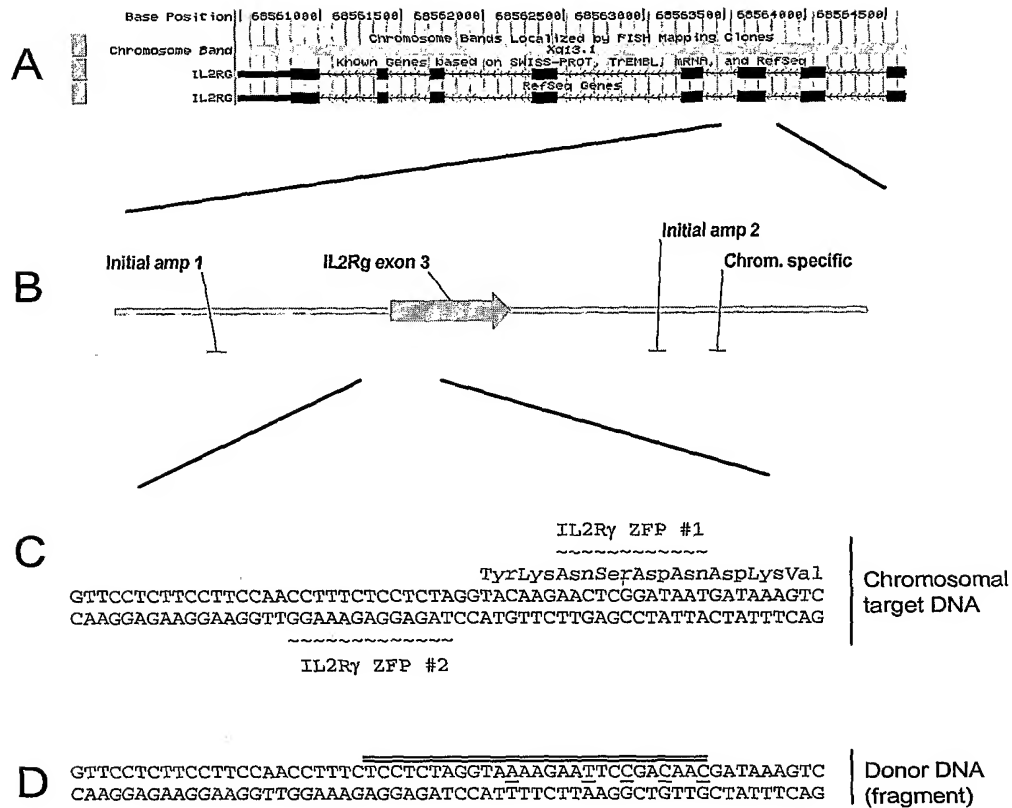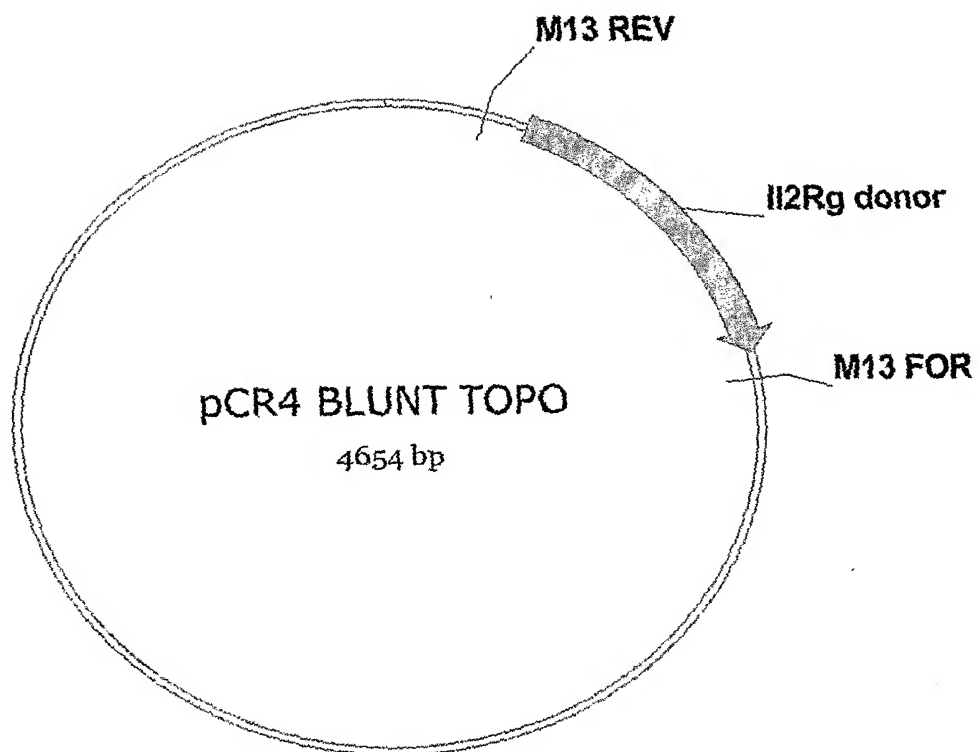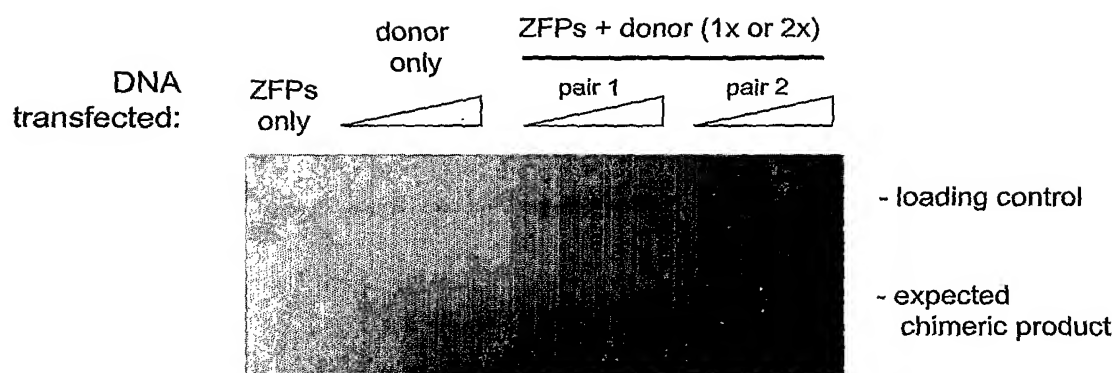
FIGURE 8

FIGURE 9

A

Base Position | 68561000| 68561500| 68562000| 68562500| 68563000| 68563500| 68564000| 68564500|
Chromosome Band | Chromosome Bands Localized by FISH Mapping Clones
Xq13.1
IL2RG | Known Genes Based on SWISS-PROT, TrEMBL, mRNA, and RefSeq
RefSeq Genes
IL2RG

B

Initial amp 1   IL2Rg exon 3   Initial amp 2
Chrom. specific

C

```
                                    IL2Rγ ZFP #1
                                    ~~~~~~~~~~~~
                              TyrLysAsnSerAspAsnAspLysVal
GTTCCTCTTCCTTCCAACCTTTCTCCTCTAGGTACAAGAACTCGGATAATGATAAAGTC      Chromosomal
CAAGGAGAAGGAAGGTTGGAAAGAGGAGATCCATGTTCTTGAGCCTATTACTATTTCAG      target DNA
                              ~~~~~~~~~~~~
                                    IL2Rγ ZFP #2
```

D

```
GTTCCTCTTCCTTCCAACCTTTCTCCTCTAGGTAAAAGAATTCCGACAACGATAAAGTC      Donor DNA
CAAGGAGAAGGAAGGTTGGAAAGAGGAGATCCATTTTCTTAAGGCTGTTGCTATTTCAG      (fragment)
```

FIGURE 10

M13 REV

Il2Rg donor

pCR4 BLUNT TOPO
4654 bp

M13 FOR

FIGURE 11

DNA transfected:

ZFPs only

donor only

ZFPs + donor (1x or 2x)

pair 1 pair 2

- loading control

- expected chimeric product
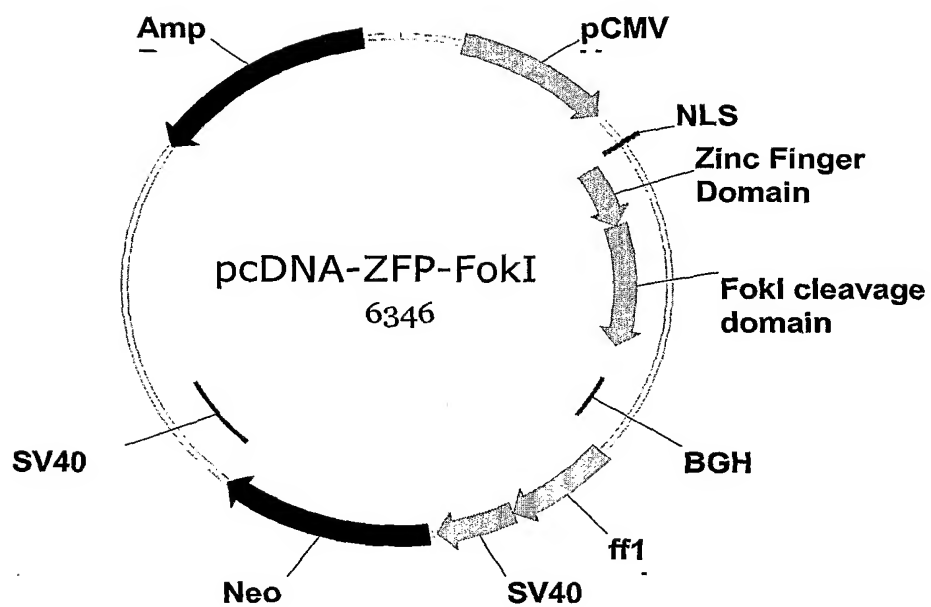
FIGURE 12

```
TAGTCCTGCAGGTTTAAACGAATTCGCCCTTTCCTCTAGGTAaAAGAAtT        50
CcGAcAAcGATAAAGTCCAGAAGTGCAGCCACTATCTATTCCCTGAAGAA       100
ATCACTTCTGGCTGTCAGTTGCAAAAAAAGGAGATCCACCTCTACCAAAC       150
ATTTGTTGTTCAGCTCCAGGACCCACGGGAACCCAGGAGACAGGCCACAC       200
AGATGCTAAAACTGCAGAATCTGGGTAATTTGGAAAGAAAGGGTCAAGAG       250
ACCAGGGATACTGTGGGACATTGGAGTCTACAGAGTAGTGTTCTTTTATC       300
ATAAGGGTACATGGGCAGAAAAGAGGAGGTAGGGGATCATGATGGGAAGG       350
GAGGAGGTATTAGGGGCACTACCTTCAGGATCCTGACTTGTCTAGGCCAG       400
GGGAATGACCACATATGCACACATATCTCCAGTGATCCCCTGGGCTCCAG       450
AGAACCTAACACTTCACAAACTGAGTGAATCCCAGCTAGAACTGAACTGG       500
AACAACAGATTCTTGAACCACTGTTTGGAGCACTTGGTGCAGTACCGGAC       550
TAAGGGCGAATTCGCGGCCGCTAAATTCAATTCGCCCTATAGTGAGTCGT       600
ATTACAATTCACTGGCCGTCGTTT
```

(SEQ ID NO:12)

FIGURE 13

```
TACTGATGGTATGGGGCCAAGAGATATATCTTAGAGGGAGGGCTGAGGGT      50
TTGAAGTCCAACTCCTAAGCCAGTGCCAGAAGAGCCAAGGACAGGTACGG     100
CTGTCATCACTTAGACCTCACCCTGTGGAGCCACACCCTAGGGTTGGCCA     150
ATCTACTCCCAGGAGCAGGGAGGGCAGGAGCCAGGGCTGGGCATAAAAGT     200
CAGGGCAGAGCCATCTATTGCTTACATTTGCTTCTGACACAACTGTGTTC     250
ACTAGCAACCTCAAACAGACACCATGGTGCATCTGACTCCTGAGGAGAAG     300
TCTGCCGTTACTGCCCTGTGGGGCAAGGTGAACGTGGATGAAGTTGGTGG     350
TGAGGCCCTGGGCAGGTTGGTATCAAGGTTACAAGACAGGTTTAAGGAGA     400
CCAATAGAAACTGGGCATGTGGAGACAGAGAAGACTCTTGGGTTTCTGAT     450
AGGCACTGACTCTCTCTGCCTATTGGTCTATTTTCCCACCCTTAGGCTGC     500
TGGTGGTCTACCCTTGGACCCAGAGGTTCTTTGAGTCCTTTGGGGATCTG     550
TCCACTCCTGATGCTGTTATGGGCAACCCTAAGGTGAAGGCTCATGGCAA     600
GAAAGTGCTCGGTGCCTTTAGTGATGGCCTGGCTCACCTGGACAACCTCA     650
AGGGCACCTTTGCCACACTGAGTGAGCTGCACTGTGACAAGCTGCACGTG     700
```

(SEQ ID NO:13)

FIGURE 14

FIGURE 15

Beta globin human genomic TARGET
700 bp

FIGURE 16

FIGURE 17

Loading Control   GC-specific Product
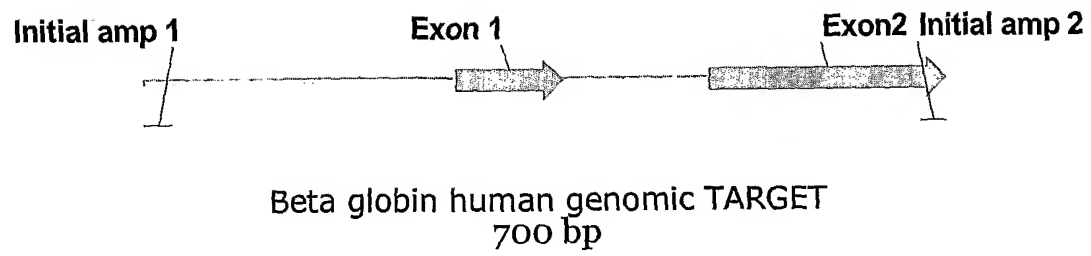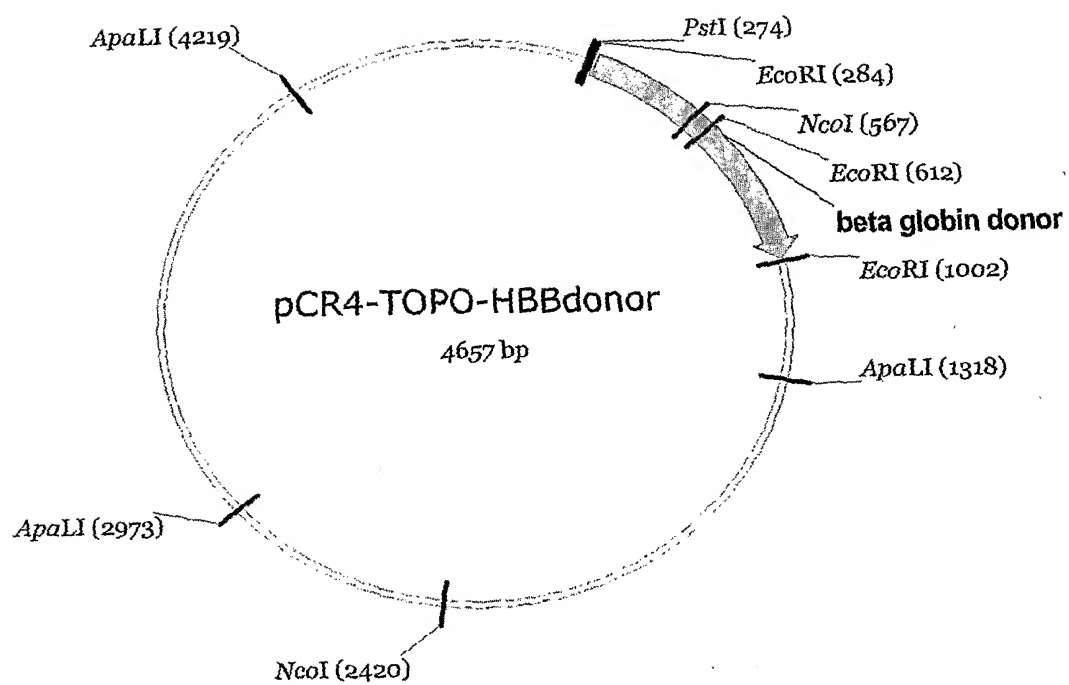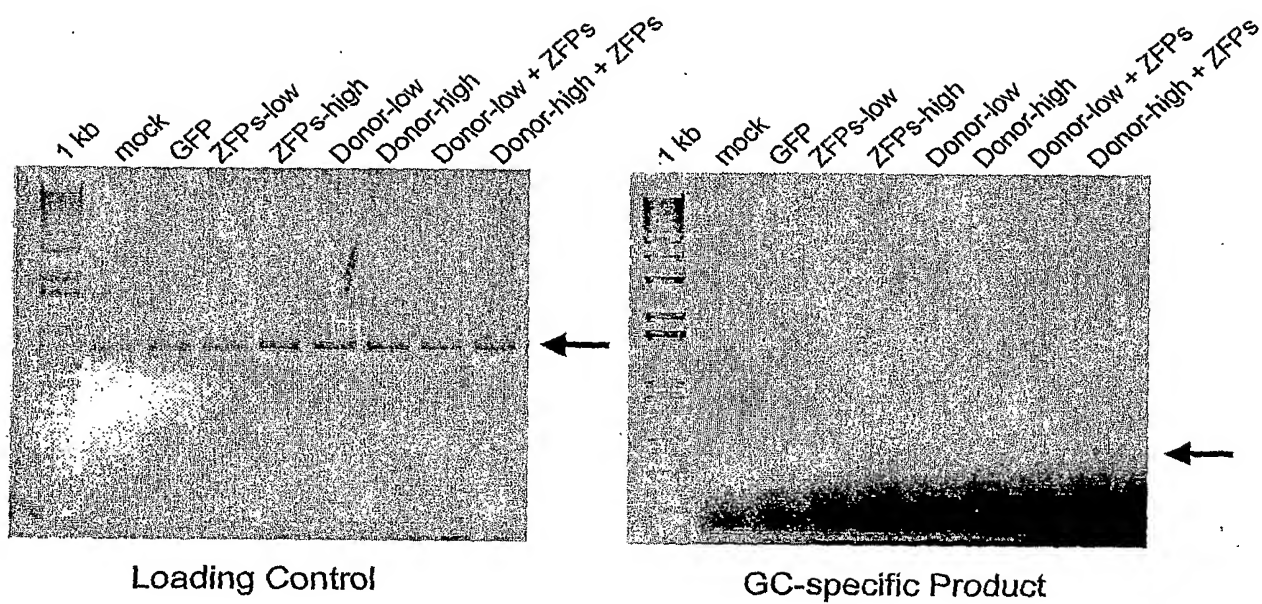
FIGURE 18

```
TGCTTACCAAGCTGTGATTCCAAATATTACGTAAATACACTTGCAAAGGA      50
GGATGTTTTTAGTAGCAATTTGTACTGATGGTATGGGGCCAAGAGATATA     100
TCTTAGAGGGAGGGCTGAGGGTTTGAAGTCCAACTCCTAAGCCAGTGCCA     150
GAAGAGCCAAGGACAGGTACGGCTGTCATCACTTAGACCTCACCCTGTGG     200
AGCCACACCCTAGGGTTGGCCAATCTACTCCCAGGAGCAGGGAGGGCAGG     250
AGCCAGGGCTGGGCATAAAAGTCAGGGCAGAGCCATCTATTGCTTACATT     300
TGCTTCTGACACAACTGTGTTCACTAGCAACCTCAAACAGACACCATGGT     350
GCATCTGACTCCTGAGGAGAAGTCTGgCGTTAgTGCCCgaattccgAtcG     400
TcAACcac
```

(SEQ ID NO:14)

FIGURE 19

```
                                          5-8
CACGTTTCGTGTTCGGAGCCGCTTTAACCCACTCTGTGGAAG
GTGCAAAGCACAAGCCTCGGCGAAATTGGGTGAGACACCTTC
   5-10
```

(SEQ ID NO:15)

FIGURE 20

```
  1 MAPKKKRKVG IHGVPAAMAE RPFQCRICMR NFSRSDNLSE HIRTHTGEKP FACDICGRKF
 61 ARNAHRINHT KIHTGSQKPF QCRICMRNFS RSDTLSEHIR THTGEKPFAC DICGRKFAAR
121 STRTTHTKIH LRQKDAARGS QLVKSELEEK KSELRHKLKY VPHEYIELIE IARNSTQDRI
181 LEMKVMEFFM KVYGYRGKHL GGSRKPDGAI YTVGSPIDYG VIVDTKAYSG GYNLPIGQAD
241 EMQRYVEENQ TRNKHINPNE WWKVYPSSVT EFKFLFVSGH FKGNYKAQLT RLNHITNCNG
301 AVLSVEELLI GGEMIKAGTL TLEEVRRKFN NGEINF
```

(SEQ ID NO:16)

FIGURE 21

```
  1 MAPKKKRKVG IHGVPAAMAE RPFQCRICMR NFSRSDSLSR HIRTHTGEKP FACDICGRKF
 61 ADSSNRKTHT KIHTGGGGSQ KPFQCRICMR NFSRSDSLSV HIRTHTGEKP FACDICGRKF
121 ADRSNRITHT KIHLRQKDAA RGSQLVKSEL EEKKSELRHK LKYVPHEYIE LIEIARNSTQ
181 DRILEMKVME FFMKVYGYRG KHLGGSRKPD GAIYTVGSPI DYGVIVDTKA YSGGYNLPIG
241 QADEMQRYVE ENQTRNKHIN PNEWWKVYPS SVTEFKFLFV SGHFKGNYKA QLTRLNHITN
301 CNGAVLSVEE LLIGGEMIKA GTLTLEEVRR KFNNGEINF
```

(SEQ ID NO:17)

FIGURE 22

CGAATTCTGCAGTCGACGGTACCGCGGGCCCGGGATCCACCGGTCGCCACC<u>ATG</u>GTGAGC
AAGGGCGAGGAGCTGTTCACCGGGGTGGTGCCCATCCTGGTCGAGCTGGACGGCGACGTA
AACGGCCACAAGTTCAGCGTGTCCGGCGAGGGCGAGGGCGATGCCACCTACGGCAAGCTG
ACCCTGAAGTTCATCTGCACCACCGGCAAGCTGCCCGTGCCCTGGCCCACCCTCGTGACC
ACCCTGACCTACGGCGTGCAGTGCTTCAGCCGCTACCCC<u>GACCACAT</u>GAAGCAGCACGAC
TTCTTCAAGTCCGCCATGCCCGAAGGCTACGTCCAGGAGCGCACCATCTTCTTCAAGGAC
GACGGCAACTACAAGACCCGCGCCGAGGTGAAGTTCGAGGGCGACACCCTGGTGAACCGC
ATCGAGCTGAAGGGCATCGACTTCAAGGAGGACGGCAACATCCTGGGGCACAAGCTGGAG
TACAACTACAACAGCCACAACGTCTATATCATGGCCGACAAGCAGAAGAACGGCATCAAG
GTGAACTTCAAGATCCGCCACAACATCGAGGACGGCAGCGTGCAGCTCGCCGACCACTAC
CAGCAGAACACCCCCATCGGCGACGGCCCCGTGCTGCTGCCCGACAACCACTACCTGAGC
ACCCAGTCCGCCCTGAGCAAAGACCCCAACGAGAAGCGCGATCACATGGTCCTGCTGGAG
TTCGTGACCGCCGCCGGGATCACTCTCGGCATGGACGAGCTGTACAAGTAAAGCGGCCGC
GACTCTAGATCATAATC

(SEQ ID NO:18)

FIGURE 23

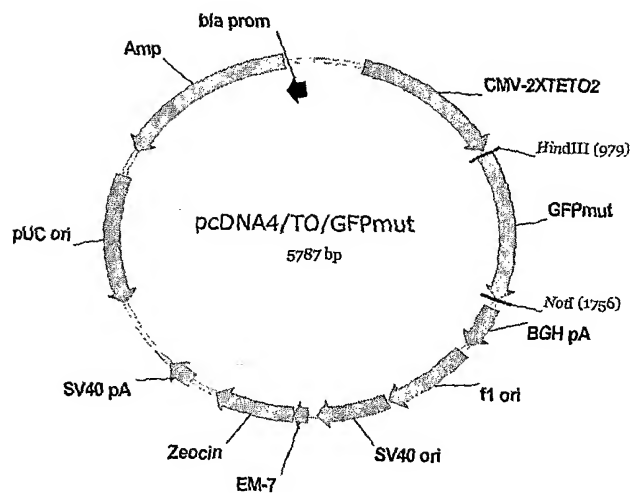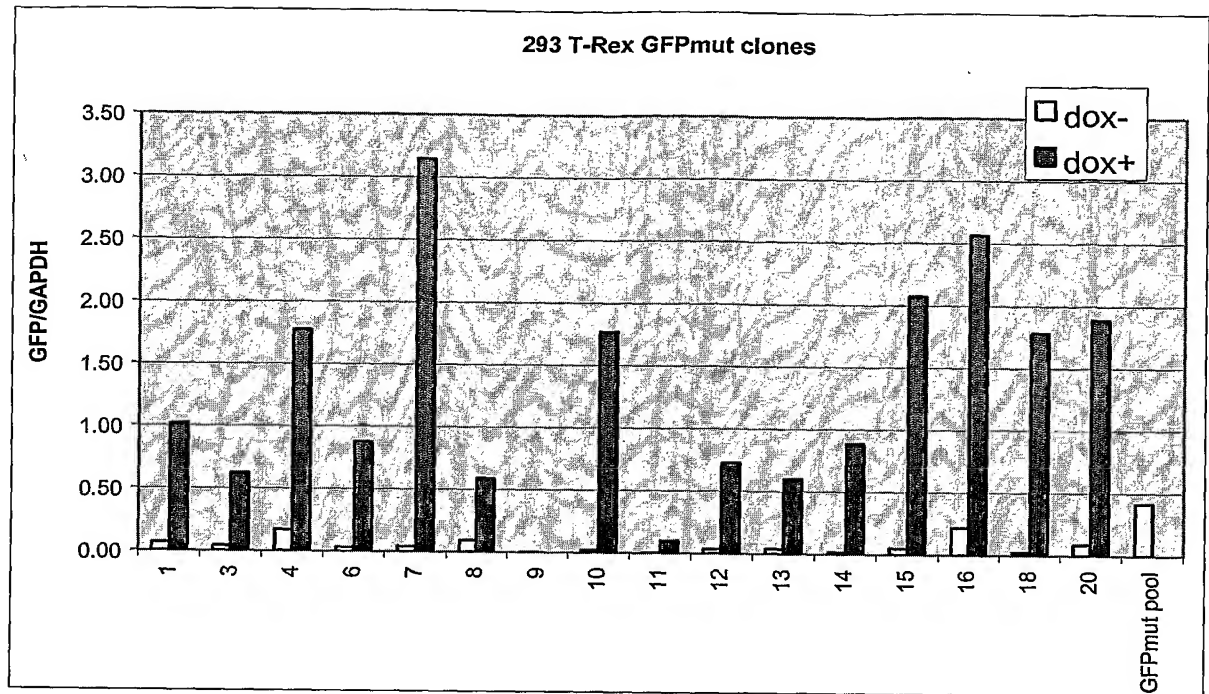CGAATTCTGCAGTCGACGGTACCGCGGGCCCGGGATCCACCGGTCGCCACCATGGTGAGC
AAGGGCGAGGAGCTGTTCACCGGGGTGGTGCCCATCCTGGTCGAGCTGGACGGCGACGTA
AACGGCCACAAGTTCAGCGTGTCCGGCGAGGGCGAGGGCGATGCCACCTACGGCAAGCTG
ACCCTGAAGTTCATCTGCACCACCGGCAAGCTGCCCGTGCCCTGGCCCACCCTCGTGACC
ACCCTGACCTACGGCGTGCAGTGCTTCAGCCGCTACCCCTAACACGAAGCAGCACGACTT
CTTCAAGTCCGCCATGCCCGAAGGCTACGTCCAGGAGCGCACCATCTTCTTCAAGGACGA
CGGCAACTACAAGACCCGCGCCGAGGTGAAGTTCGAGGGCGACACCCTGGTGAACCGCAT
CGAGCTGAAGGGCATCGACTTCAAGGAGGACGGCAACATCCTGGGGCACAAGCTGGAGTA
CAACTACAACAGCCACAACGTCTATATCATGGCCGACAAGCAGAAGAACGGCATCAAGGT
GAACTTCAAGATCCGCCACAACATCGAGGACGGCAGCGTGCAGCTCGCCGACCACTACCA
GCAGAACACCCCCATCGGCGACGGCCCCGTGCTGCTGCCCGACAACCACTACCTGAGCAC
CCAGTCCGCCCTGAGCAAAGACCCCAACGAGAAGCGCGATCACATGGTCCTGCTGGAGTT
CGTGACCGCCGCCGGGATCACTCTCGGCATGGACGAGCTGTACAAGTAAAGCGGCCGCGA
CTCTAGATCATAATC

(SEQ ID NO:19)

FIGURE 24
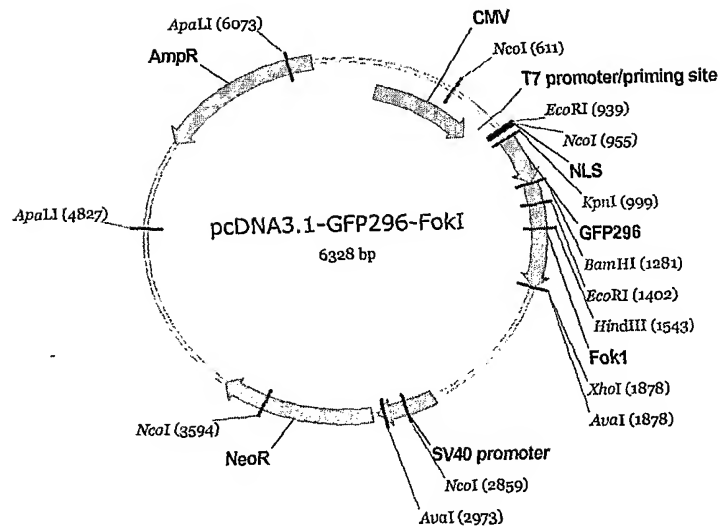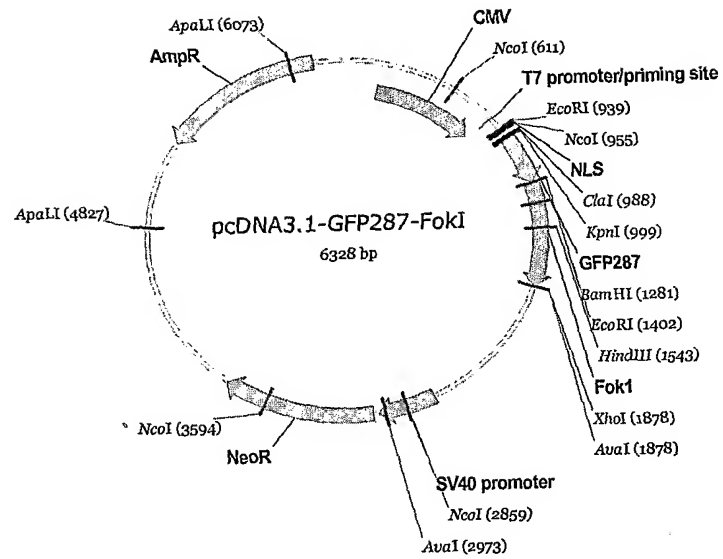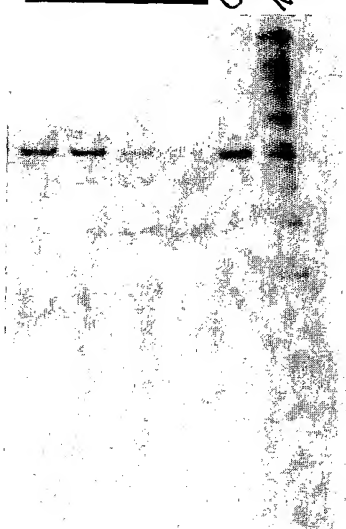
FIGURE 25

FIGURE 26

FIGURE 27

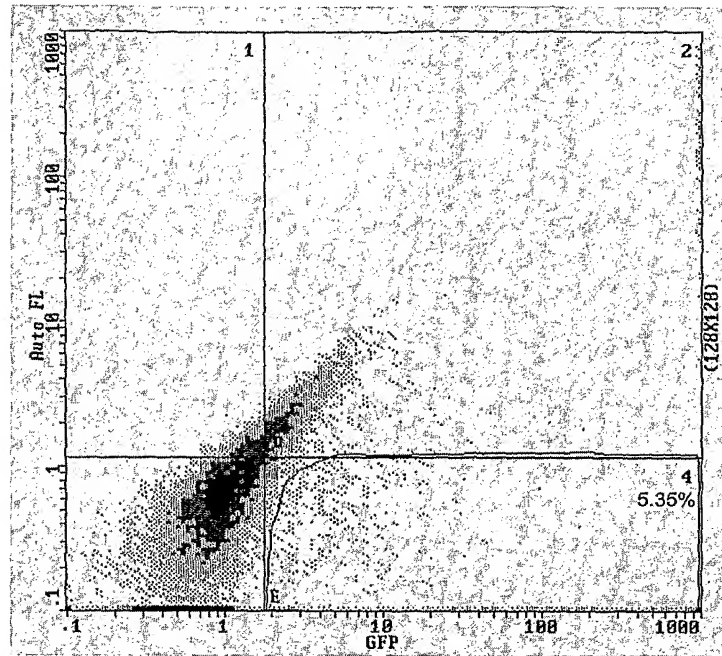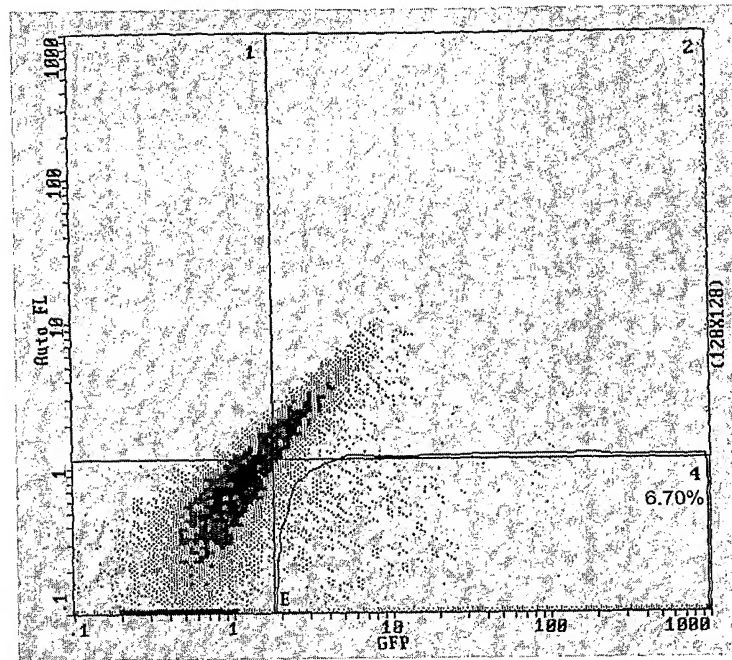

293 T-Rex GFPmut clones
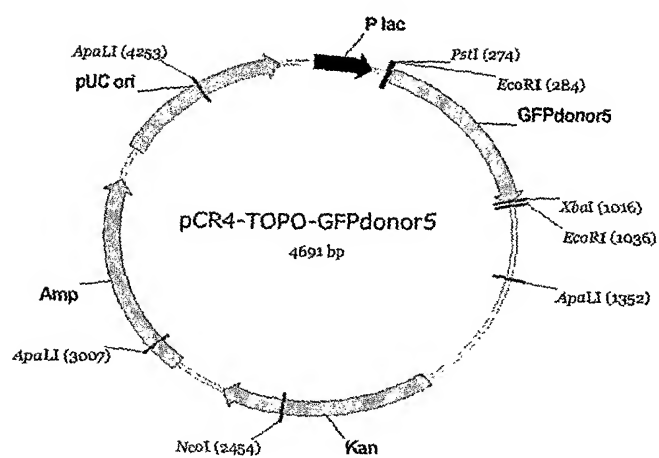
FIGURE 28

FIGURE 29

```
GGCGAGGAGCTGTTCACCGGGGTGGTGCCCATCCTGGTCGAGCTGGACGGCGACGTAAAC
GGCCACAAGTTCAGCGTGTCCGGCGAGGGCGAGGGCGATGCCACCTACGGCAAGCTGACC
CTGAAGTTCATCTGCACCACCGGCAAGCTGCCCGTGCCCTGGCCCACCCTCGTGACCACC
CTGACCTACGGCGTGCAGTGCTTCAGCCGCTACCCCGACCACATGAAGCAGCACGACTTC
TTCAAGTCCGCCATGCCCGAAGGCTACGTCCAGGAGCGCACCATCTTCTTCAAGGACGAC
GGCAACTACAAGACCCGCGCCGAGGTGAAGTTCGAGGGCGACACCCTGGTGAACCGCATC
GAGCTGAAGGGCATCGACTTCAAGGAGGACGGCAACATCCTGGGGCACAAGCTGGAGTAC
AACTACAACAGCCACAACGTCTATATCATGGCCGACAAGCAGAAGAACGGCATCAAGGTG
AACTTCAAGATCCGCCACAACATCGAGGACGGCAGCGTGCAGCTCGCCGACCACTACCAG
CAGAACACCCCCATCGGCGACGGCCCCGTGCTGCTGCCCGACAACCACTACCTGAGCACC
CAGTCCGCCCTGAGCAAAGACCCCAACGAGAAGCGCGATCACATGGTCCTGCTGGAGTTC
GTGACCGCCGCCGGGATCACTCTCGGCATGGACGAGCTGTACAAGTAAAGCGGCCGCGAC
TCTAGATCATAATC
```

(SEQ ID NO:20)

FIGURE 30

FIGURE 31

FIGURE 32

```
GGCGAGGAGCTGTTCACCGGGGTGGTGCCCATCCTGGTCGAGCTGGACGGCGACGTAAAC
GGCCACAAGTTCAGCGTGTCCGGCGAGGGCGAGGGCGATGCCACCTACGGCAAGCTGACC
CTGAAGTTCATCTGCACCACCGGCAAGCTGCCCGTGCCCTGGCCCACCCTCGTGACCACC
CTGACCTACGGCGTGCAGTGCTTCAGCCGCTACCCCGACCACATGAAGCAGCACGACTTC
TTCAAGTCCGCCATGCCCGAAGGCTACGTCCAGGAGCGCACCATCTTCTTCAAGGACGAC
GGCAACTACAAGACCCGCGCCGAGGTGAAGTTCGAGGGCGACACCCTGGTGAACCGCATC
GAGCTGAAGGGCATCGACTTCAAGGAGGACGGCAACATCCTGGGGCACAAGCTGGAGTAC
AACTACAACAGCCACAACGTCTATATCATGGCCGACAAGCAGAAGAACGGCATCAAGGTG
AACTTCAAGATCCGCCACAACATCGAGGACGGCAGCGTGCAGCTCGCCGACCACTACCAG
CAGAACACCCCCATCGGCGACGGCCCCGTGCTGCTGCCCGACAACCACTACCTGAGCACC
CAGTCCGCCCTGAGCAAAGACCCCAACGAGAAGCGCGATCACATGGTCCTGCTGGAGTTC
GTGACCGCCGCCGGGATCACTCTCGGCATGGACGAGCTGTACAAGTAAAGCGGCCGCTCG
AGTCTAGAGGGCCCGTTTAAACCCGCTGATCAGCCTCGACTGTGCCTTCTAGTTGCCAGC
CATCTGTTGTTTGCCCCTCCCCCGTGCCTTCCTTGACCCTGGAAGGTGCCACTCCCACTG
TCCTTTCCTAATAAAATGAGGAAATTGCATCGCATTGTCTGAGTAGGTGTCATTCTATTC
TGGGGGGTGGGGTGGGGCAGGACAGCAAGGGGGAGGATTGGGAAGACAATAGCAGGCATG
CTGGGGATGCGGTGGGCTCTATGGCTTCTGAGGCGGAAAGAACCAGCTGGGGCTCTAGGG
GGTATCCCCACGCGCCCTGTAGCGGCGCATTAAGCGCGGCGGGTGTGGTGGTTACGCGCA
GCGTGACCGCTACACTTGCCAGCGCCCTAGCGCCCGCTCCTTTCGCTTTCTTCCCTTCCT
TTCTCGCCACGTTCGCCGGCTTTCCCCGTCAAGCTCTAAATCGGGGGCTCCCTTTAGGGT
TCCGATTTAGTGCTTTACGGCACCTCGACCCCAAAAAACTTGATTAGGGTGATGGTTCAC
GTAGTGGGCCATCGCCCTGATAGACGGTTTTTCGCCCTTTGACGTTGGAGTCCACGTTCT
TTAATAGTGGACTCTTGTTCCAAACTGGAACAACACTCAACCCTATCTCGGTCTATTCTT
TTGATTTATAAGGGATTTTGCCGATTTCGGCCTATTGGTTAAAAAATGAGCTGATTTAAC
AAAAATTTAACGCGAATTAATTCTGTGGAATGTGTGTCAGTTAGGGTGTGGAAAGTCCCC
AGGCTCCCCAGCAGGCAGAAGTATGCA
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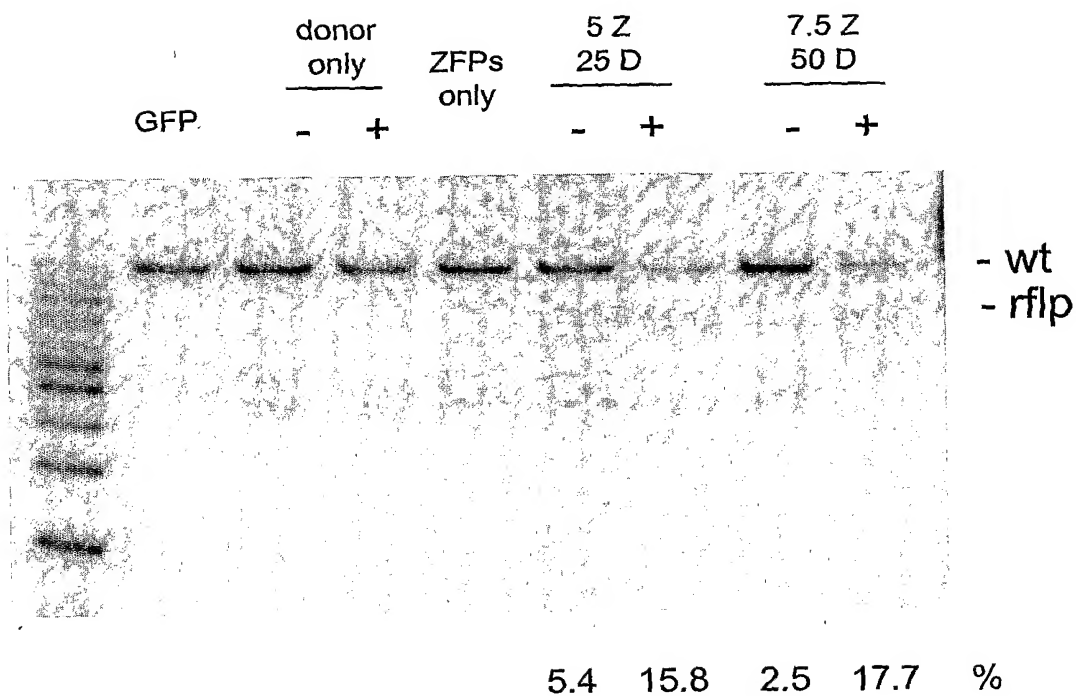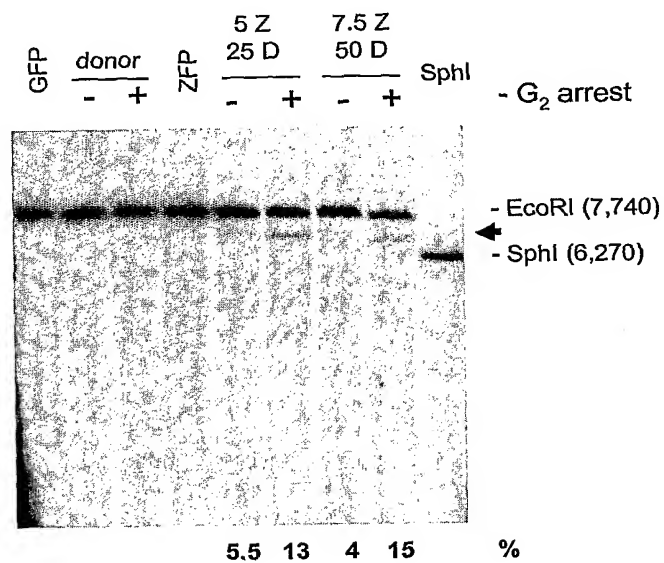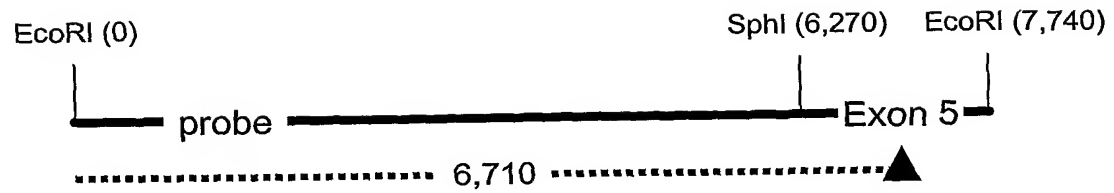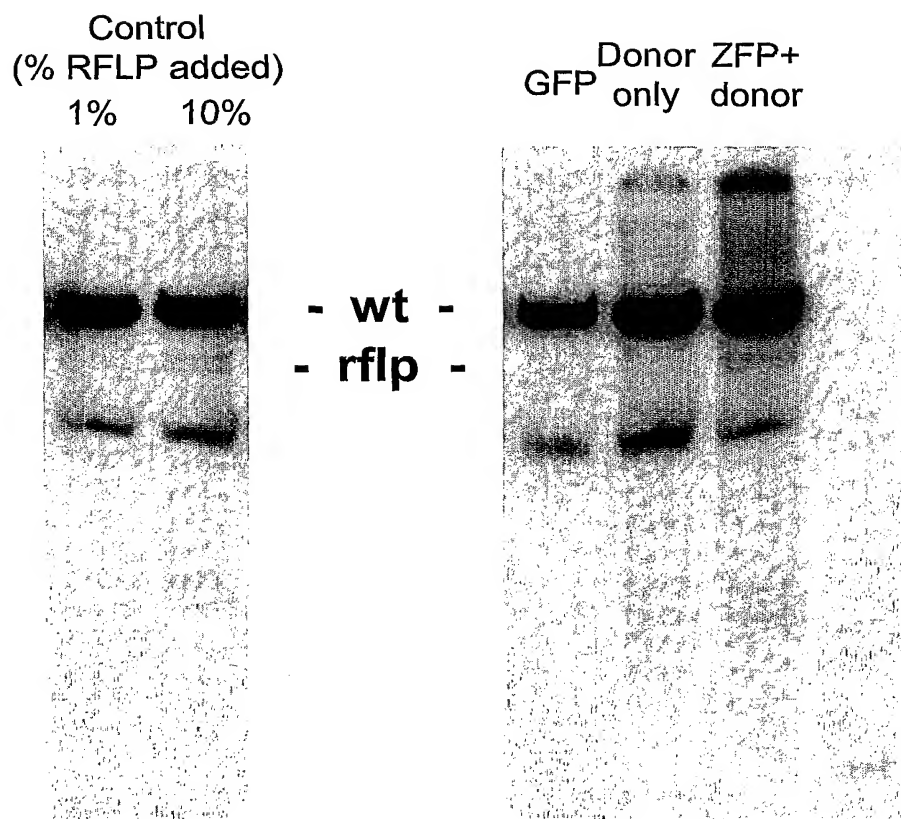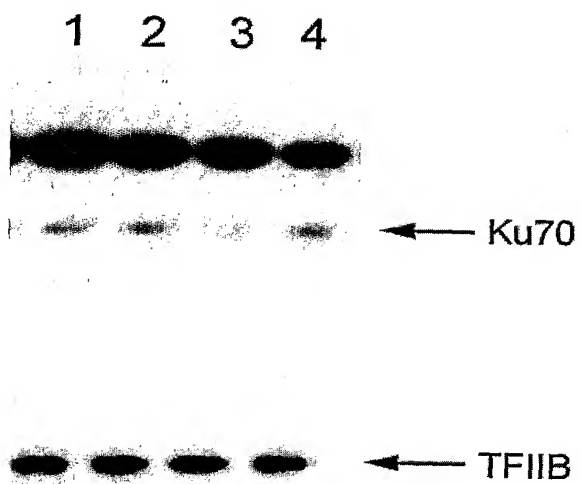
(SEQ ID NO:21)

FIGURE 33
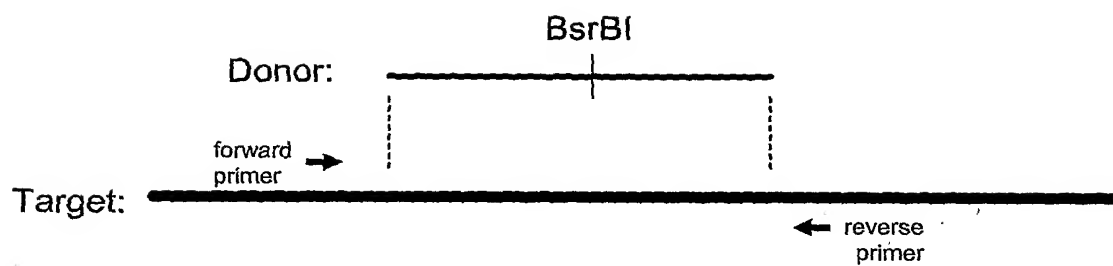
FIGURE 34
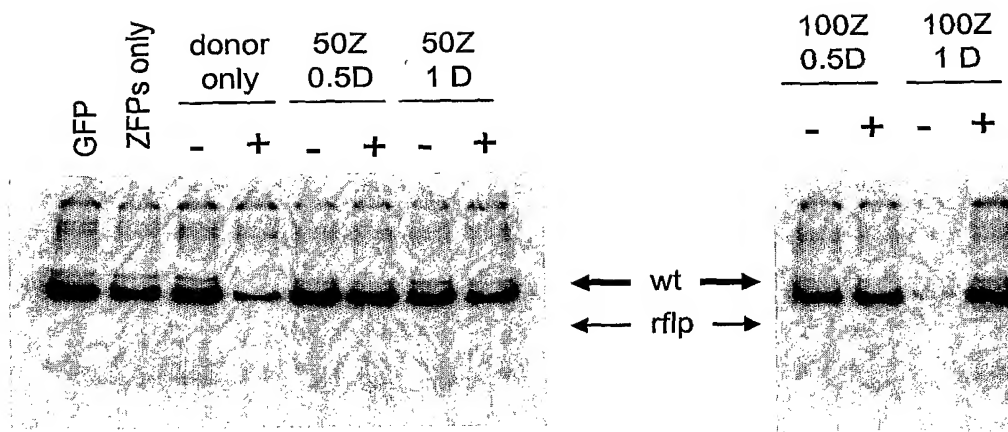
FIGURE 35
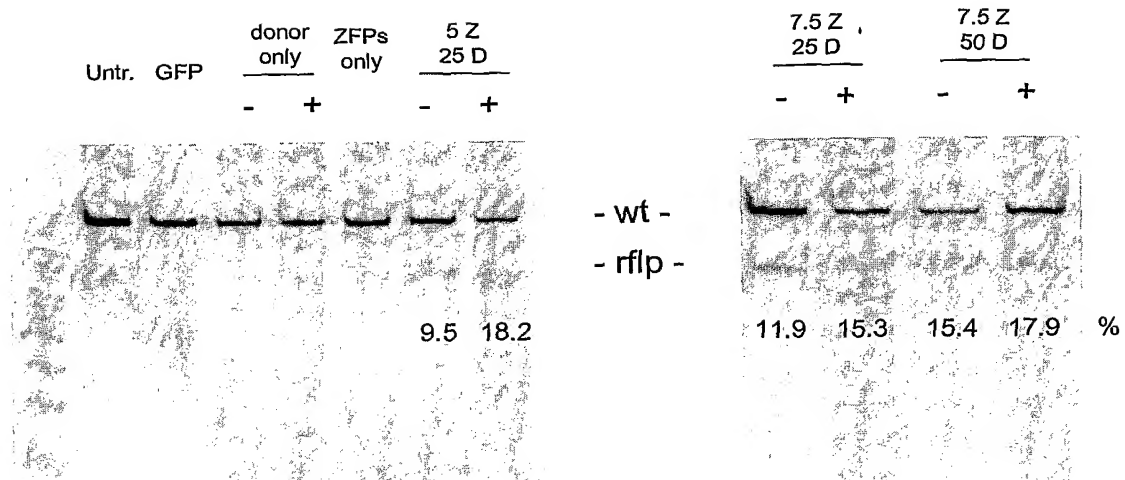
FIGURE 36

FIGURE 37

FIGURE 38

FIGURE 39

FIGURE 40

sca-29b:

MAERPFQCRICMRNFSQSGDLTRHIRTHTGEKPFACDICGRKFATSANLSRHTK
IHTGGGGSQKPFQCRICMRNFSDRSALSRHIRTHTGEKPFACDICGRKFAQSG
HLSRHTKIH (SEQ ID NO:22)

sca-36a:

MAERPFQCRICMRNFSRSQTRKTHIRTHTGEKPFACDICGRKFAQKRNRTKHT
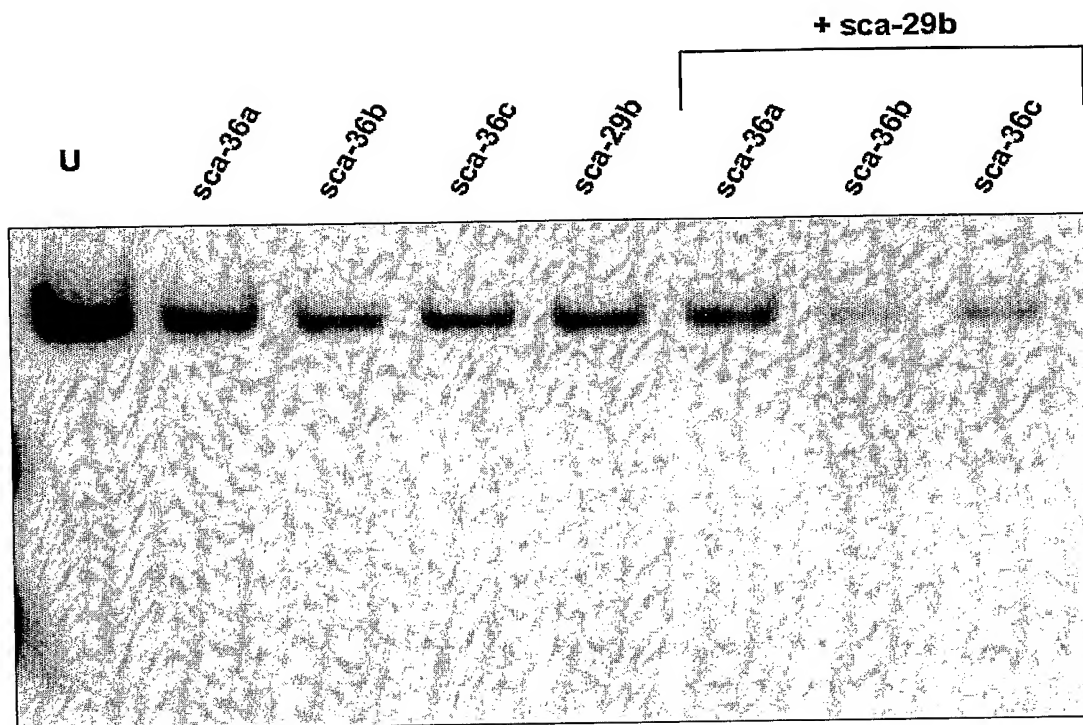KIHTGSQKPFQCRICMRNFSDRSALSRHIRTHTGEKPFACDICGRKFAQSGNLA
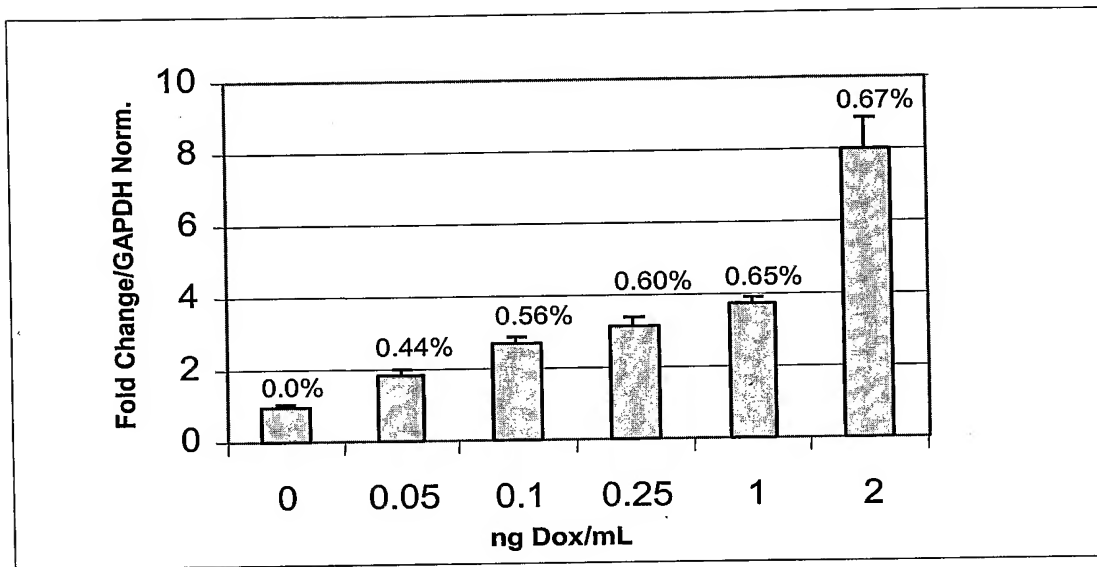RHTKIH (SEQ ID NO:23)

sca-36b:

MAERPFQCRICMRNFSTSGSLSRHIRTHTGEKPFACDICGRKFADRSDLSRHTK
IHTGGGGSQKPFQCRICMRNFSDRSALSRHIRTHTGEKPFACDICGRKFAQSG
NLARHTKIH (SEQ ID NO:24)
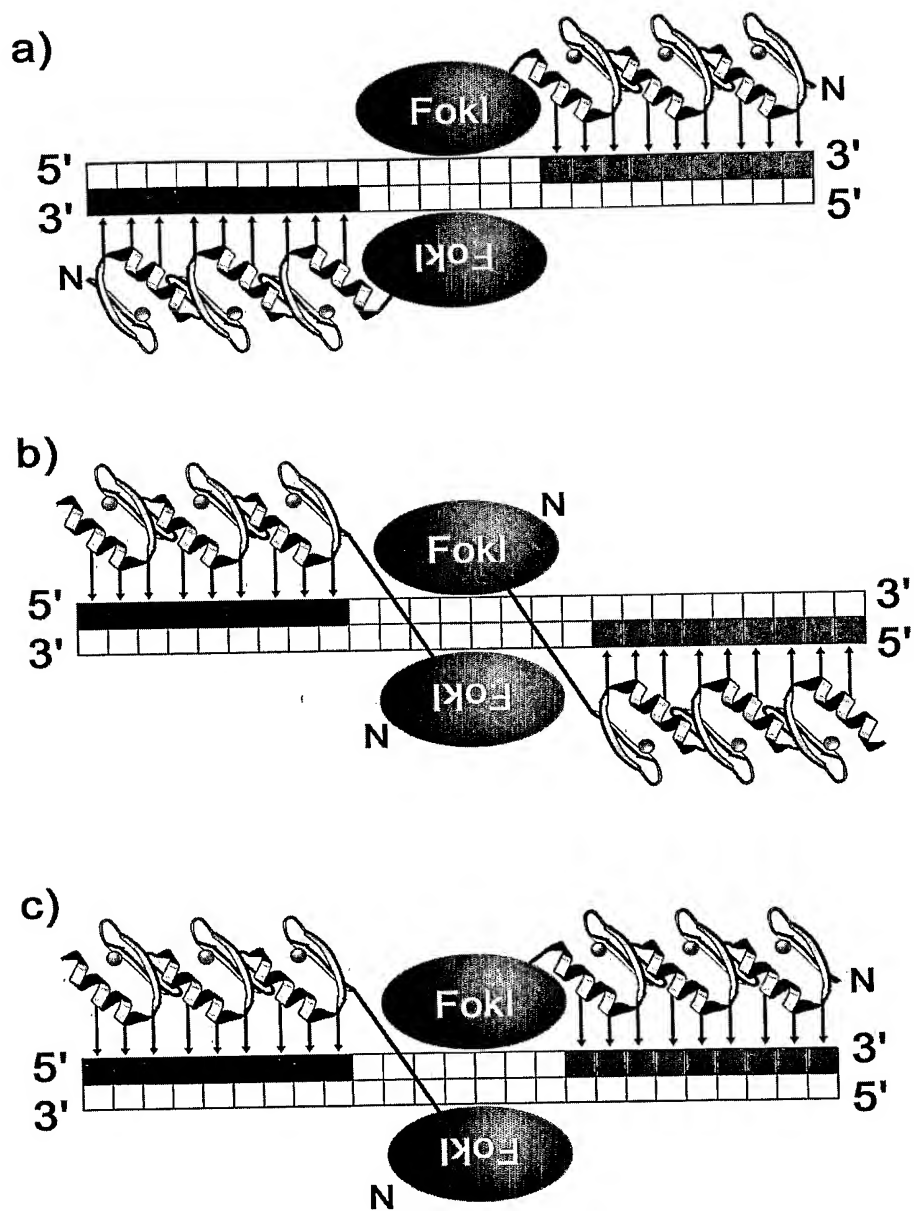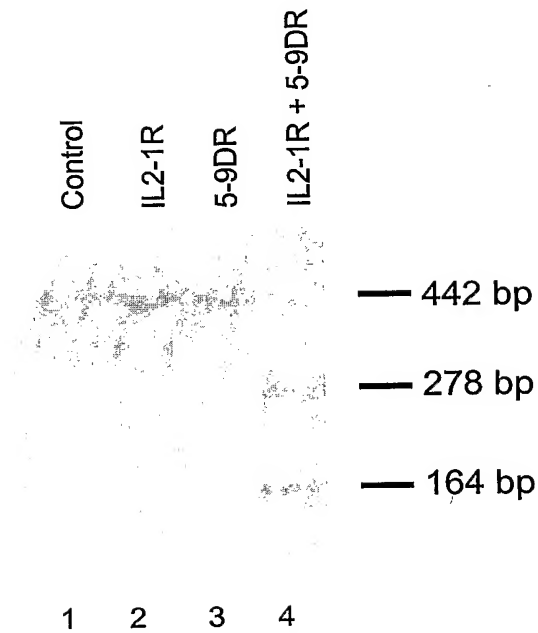
sca-36c:

MAERPFQCRICMRNFSTSSSLSRHIRTHTGEKPFACDICGRKFADRSDLSRHTK
IHTGGGGSQKPFQCRICMRNFSDRSALSRHIRTHTGEKPFACDICGRKFAQSG
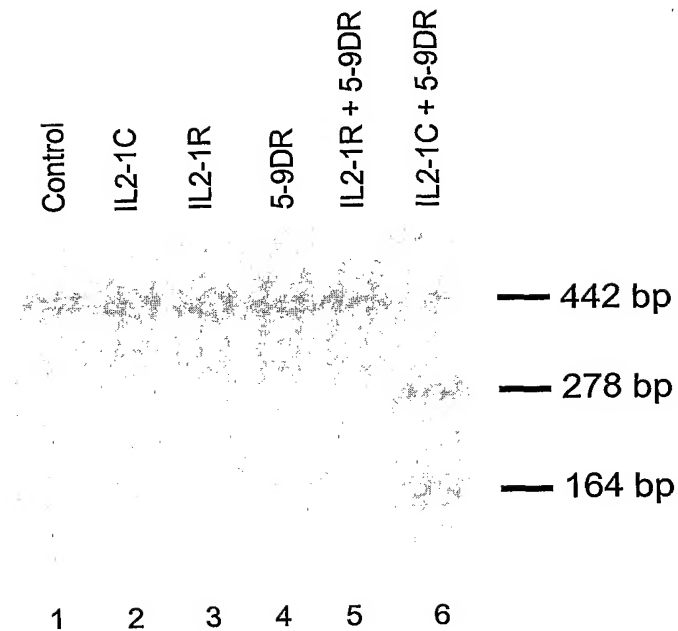NLARHTKIH (SEQ ID NO:25)

FIGURE 41

FIGURE 42

FIGURE 43

FIGURE 44


```
              Control  IL2-1R  5-9DR  IL2-1R + 5-9DR

                                              —— 442 bp

                                              —— 278 bp

                                              —— 164 bp

               1       2       3       4
```

```
                  59D
      -tAAAGCGGCTCCGaaccacATTATCCGAGTTc-
      -aTTTCGCCGAGGCttggtgTAATAGGCTCAAg-
                              IL2-1
```

(SEQ ID NO: 211)

FIGURE 45

Lane	1	2	3	4	5	6
Label	Control	IL2-1C	IL2-1R	5-9DR	IL2-1R + 5-9DR	IL2-1C + 5-9DR

— 442 bp

— 278 bp

— 164 bp

```
       59D                    IL2-1
-tAAAGCGGCTCCGaaccacAACTCGGATAATg-
-aTTTCGCCGAGGCttggtgTTGAGCCTATTAc-
```

(SEQ ID NO: 212)